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NIPIGON BAY: AN ASSESSMENT OF THE ACUTE TOXICITY, GROWTH IMPAIRMENT AND FLESH TAINTING POTENTIAL OF A BLEACHED KRAFT MILL EFFLUENT (BKME) ON RAINBOW TROUT (*Salmo gairdneri*)

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Ministry of the Environment

Ontario

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Deputy Minister

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by

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SUMMARY

Static and continuous-flow bioassays utilizing juvenile rainbow trout (*Salmo gairdneri*) were conducted on a northern Ontario bleached kraft mill effluent to assess the range of acute toxicity and related sublethal effects. The acute lethality of the total mill effluent, as determined by static bioassay procedures, ranged from a 96 hr. LC_{50} value of 14 to 49.0% v/v. The results of these bioassays depended on testing methodology and effluent treatment systems employed by the mill when samples were collected. Continuous-flow bioassays conducted on the effluent produced 96-hr. LC_{50} values of 21.8 and 24.8%.

Growth rate of juvenile rainbow trout (1-2 gm) held under continuous-flow conditions for 18 days was significantly reduced at 6% v/v effluent concentration (ρ 0.05). Three percent v/v effluent, the lowest concentration impairing the flavour of yearling rainbow trout after a 48 hr. exposure, represented 0.14 of the incipient 96 hr. continuous-flow LC_{50}. Increased exposure (144 hours) of trout to the next lowest concentration (2% v/v) did not impair flesh flavour.

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INTRODUCTION

Within the framework of the I.J.C. Upper Lakes Reference group, studies were undertaken jointly by several sections of the Ontario Ministry of the Environment, as well as the Inland Waters Directorate and Great Lakes Biolimnology Laboratory of Environment Canada. The purpose of the project was to provide further knowledge in regard to the fate and effect of contaminants entering the Upper Great Lakes from an industrial point source discharge. The particular site chosen for this purpose was the Domtar Packaging Limited mill, located at Red Rock, Nipigon Bay, Lake Superior.

The Domtar mill employs a mixed groundwood kraft pulping operation with an annual production capacity of 200,000 t of kraft linerboard and 60,000 t of newsprint. Wood chips utilized in the pulping operation are comprised of 90% softwoods and 10% hardwoods.

Main production areas within the mill include a woodroom, groundwood plant, semi-chemical pulp plant, Kraft pulp plant, two Fourdrinier paper machines and a bleach plant. The bleach plant operation utilizes the three stage process of chlorination, caustic extraction and chlorine dioxide extraction.

The mill operates continuously except for the semi-chemical pulp plant and the bleach plant. These two production areas operate intermittently as dictated by production requirements.

Domtar Packaging Limited is the major consumer of water from Nipigon Bay with a mean daily consumption of about 9.6×10^7 litres/day (21.1 m.i.g.d.). The only other major consumers in the area are the town of Nipigon and

Improvement District of Red Rock. They draw their domestic water supplies from the Nipigon River and Nipigon Bay respectively.

Domtar Packaging Limited discharges approximately 9.5×10^7 litres of effluent per day in the northwest portion of Nipigon Bay (Fig. 1). This effluent includes the untreated sanitary wastes from the Improvement District of Red Rock (population 1896) (Canada Department of the Environment, 1973).

At present, approximately 60% of the total mill effluent is passed through a 45 meter diameter primary clarifier resulting in an average 80% removal of suspended material. Effluents treated by this facility originate from the woodroom, groundwood plant, Kraft pulp plant washers and digestors, newsprint paper machine and liner board paper machine. Prior to the installation of this facility in the fall of 1972 there was no system for the removal of suspended solids.

A turpentine recovery system was installed in 1972, with an average daily yield of approximately 7 litres per air dried ton of paper produced, and a foul gas condensate steam stripping facility was installed early in 1975. Additional pollution abatement facilities that are scheduled for construction include a system for the collection of black liquor spills and an increased capacity for the recovery furnace.

Construction of a primary treatment facility for the sanitary wastes originating from the Improvement District of Red Rock is scheduled for completion in 1977. Major discharges upstream of the Domtar outfall originate from the town of Nipigon (population 2,629) and include the town's primary sewage treatment facility, rated at 1.1×10^6 litres/day (0.25 m.i.g.d.), and the Multiply Plywood Company.

Several authors (Alderdice & Brett, 1957 and Gordon & Servizi, 1974)

have described the toxicity to salmonids of bleached kraft mill effluents discharged by coastal mills to marine and estuarine environments. Howard & Walden (1965) and Sprague & McLeese (1968), utilizing salmonids as the test species, determined 96-h LC50 values of 9.3% and 12-15%, respectively, for unneutralized bleached kraft effluents from interior mills. However, only a limited amount of data is available on the toxicity of effluents discharged by Ontario mills to freshwater systems. The difference in tree species pulped by British Columbia mills -- sitka spruce, western hemlock, lodgepole pine and Douglas fir as compared to jackpine, spruce, poplar and birch for Ontario mills -- undoubtedly affects the toxicity of the effluent.

The Canadian federal effluent guidelines (Canada Department of the Environment, 1971) assess discharges from the pulp and paper industry based on acute lethality to rainbow trout (*Salmo gairdneri*). These guidelines stipulate that no degradation of water quality shall result from pulp mill discharges. Ontario provincial statutes (1974) maintain that no discharge shall cause injury or damage to plant or animal life. Interpretation of the above legislation requires that the more sensitive sublethal response of aquatic organisms be considered as the ultimate test when establishing effluent standards rather than relying solely on acute lethality to rainbow trout.

Reduced growth rates and impairment of flesh flavor of salmonids exposed to low concentrations (<10%) of kraft pulp mill effluents have been described by Warren *et al*. (1973) and Cook *et al*. (1971).

In the past, Nipigon Bay has supported a large commercial fishery with walleye (*Stizostedion vitreum*) considered the major species. However, more recently, German (1968) reported tainting in the flesh of walleye taken from Nipigon Bay and resultant market rejections of these fish.

Domtar Packaging Limited installed a condensate steam stripper to treat digester blow gases and evaporator condensates in an attempt to reduce the acute lethality and flesh tainting potential of the total mill effluent. The steam stripping facility was expected to remove volatiles such as hydrogen sulphide, methyl mercaptan, disulphides, methanol, ethanol, acetone, methyl ethyl ketone and terpenes. Previous studies (Cook et al. 1971; Seppovaara & Hynninen 1970) indicated that these compounds were responsible for a large portion of the toxicity and tainting of a pulp and paper effluent and could be effectively removed by steam stripping.

The objective of this particular study was to determine the range of acute lethality and growth impairment of a northern Ontario bleached kraft mill effluent to juvenile rainbow trout. Upon installation of the steam stripper an additional study was conducted to investigate the occurrence of flavor impairment of the fish flesh after exposure to low concentrations of the total mill effluent.

To determine the most representative procedure for evaluating kraft mill effluent acute toxicity, 96-h static, semi-static (48-h solution replacement) and continuous-flow bioassays were conducted.

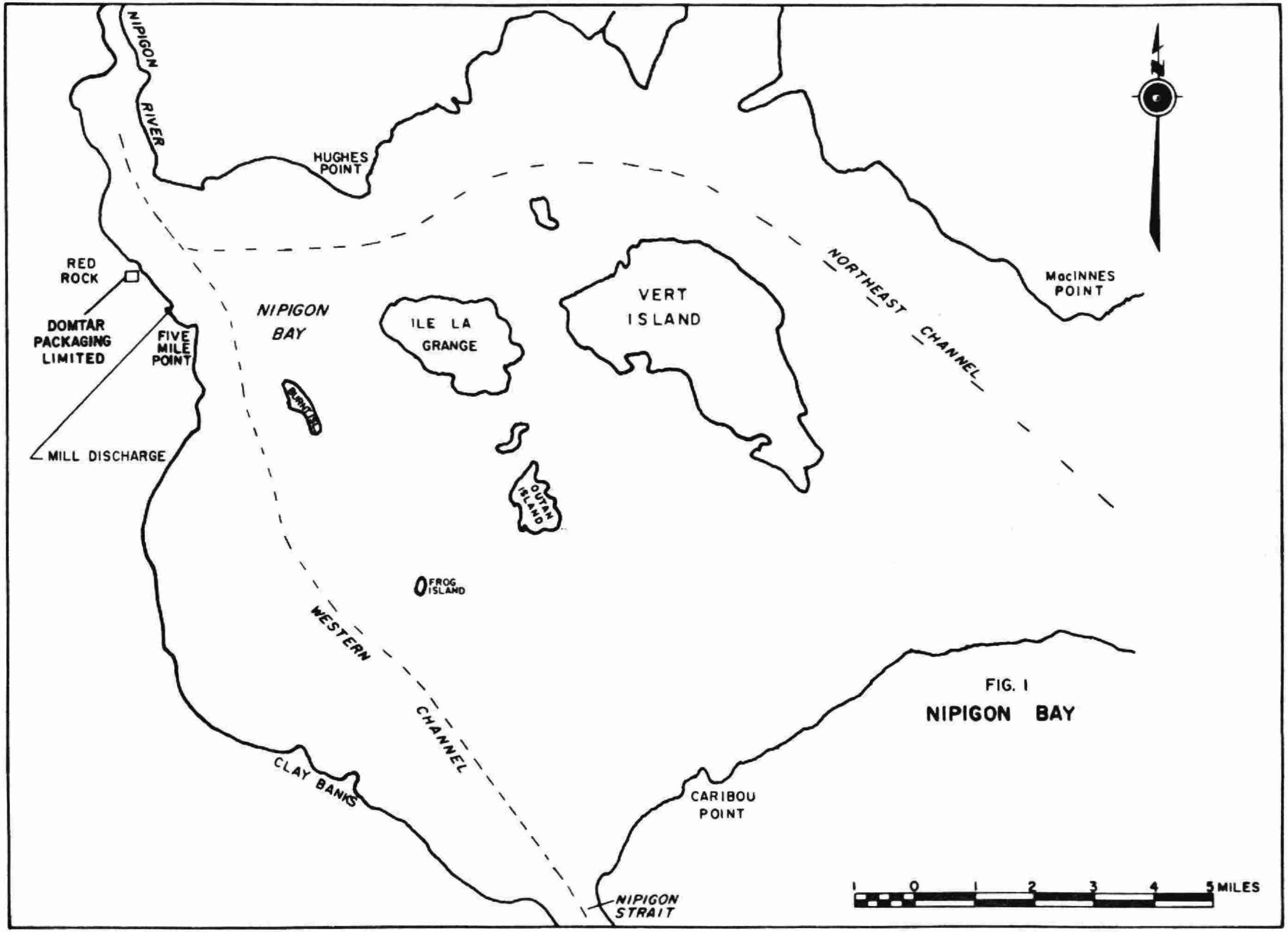


FIG. 1
NIPIGON BAY

MATERIALS AND METHODS

Rainbow trout (<u>Salmo gairdneri</u>), 1-2 g in size, were purchased from a commercial hatchery near Otterville, Ontario and were acclimated on site to the dilution water at 15°C for a minimum of 2 weeks prior to use. Mortality was less than 1% for any one week prior to testing. All fish were starved for 24 h prior to testing.

All bioassays were conducted in a prefabricated building adjacent to the mill's combined effluent channel. Effluent was pumped from this channel at a point approximately 80 m upstream of the discharge to Nipigon Bay.

Results of three preliminary range tests indicated that an LC50 for the total mill effluent was between 10% and 50% v/v. Subsequent effluent concentrations for acute bioassays were chosen based on these results and operating conditions of the mill at the time of sample collection for each bioassay. All concentrations were tested in duplicate and control fish were exposed to dilution water pumped from a submerged (10 metre) intake in Nipigon Bay.

Acute static and continuous-flow bioassays were conducted over a 96-h period and pH, temperature, conductivity and dissolved oxygen content of the test solutions were measured daily. Mortalities were recorded at 0.5, 1, 3, 6, 12, 24, 36, 48, 72 and 96 h. Death of a fish was recorded as lack of opercular ventilation and failure to respond to prodding. Dead fish were removed at once and fork length and wet weight were measured.

Bioassay data were plotted on log-probability paper and 96-h LC50 values were estimated.

Acute Bioassays - Static

Effluent samples for each static bioassay were obtained by compositing grab samples taken over a 1-h period when the mill was operating under normal conditions. Of the seven static bioassays conducted, five were carried out on site at the mill while effluent samples for two additional bioassays were transported to a central laboratory for evaluation.

On-site testing was initiated within 12 h of collection of the sample. The effluent samples transported to the central laboratory were tested within 36 h. All samples were maintained at 4°C in sealed 20-litre containers until testing. Effluent samples were neither filtered nor neutralized.

Ten-litre test solutions (6-32% v/v) were prepared in polyethylene containers and placed in a water bath for temperature regulation. Samples tested on site were diluted with water from Nipigon Bay, whereas testing at the central laboratory utilized dechlorinated municipal tap water for dilution (Appendix 1). Test temperatures were 15 ± 1°C on site and 12 ± 1°C at the central laboratory. Five fish were randomly placed in each of the effluent concentrations to give a maximum fish loading density of 1.09 g/litre.

Samples tested on site were renewed after 48 h. Fresh dilutions were prepared from the original effluent composite and the dilution water for the replacement solutions was vigorously aerated with oil-free air prior to mixing with the effluent. On-site static bioassays were aerated with pure oxygen at 250 ml/min for 2 min after 24 and 72 h. Pre- and post-treatment dissolved oxygen concentrations were measured on each of these occasions. Effluent samples tested at the central laboratory were continuously aerated but there was no solution replacement. All other bioassay

procedures conformed to the guidelines as set out in Standard Methods for the Examination of Water and Wastewater (APHA 1971). Prior to each static bioassay, samples of dilution water and effluent were subjected to routine water quality analysis. The results of chemical analysis of total mill effluent samples collected during the static bioassay series, as well as other effluent variables and flow rates as provided by mill personnel are presented in Appendix 2.

Acute Bioassays - Continuous-Flow

Effluent for continuous-flow bioassays was continuously pumped from the mill's main effluent channel and was maintained at 15 ± 1°C by passing it through 18.3 m of 0.9 cm bore stainless steel tubing immersed in a 1350-litre 15°C water bath. The cooled effluent was shunted to a modified proportional diluter (Mount and Brungs 1967) every 2 min, thus exposing the fish to the full range of waste quality resulting from production changes within the mill.

The diluter supplied each of the twelve 100-litre glass bioassay vessels at the rate of 265 ± 20 ml per min producing a 90% molecular replacement in 15 h. Test solutions were not aerated. Ten fish were randomly assigned to each of the effluent concentrations and control solutions after the bioassay vessels had attained their selected concentrations.

A commercial monitoring system (Leeds & Northrup) recorded the pH and conductivity of the whole effluent prior to dilution throughout the 96-h exposure period to detect spills.

Samples were taken from each of the effluent concentrations and the control solutions when the fish were placed in the bioassay vessels and again at 48 h. These samples, collected at 0, 48 and 96 h test time

periods, were subjected to routine water quality analysis and resin acid determinations.

Sublethal Effects - Growth Impairment

The study on growth impairment was undertaken prior to the installation of the steam stripper treatment facility at the mill.

Juvenile rainbow trout were acclimated on site to dilution water and test temperature (15 ± 1°C) for a 2-wk period with no significant mortality (< 1%).

Prior to exposure, the fish were starved for 24 h, selected for uniform size, lightly anesthetized with tricaine methanesulfonate (MS-222 Sandoz) and weighed to the nearest 0.01 g. Fork length was measured to the nearest 0.1 cm. Twenty fish, about 1.3 g, were randomly placed in each of the twelve 100-litre growth aquaria to provide a duplicate population for each of the six treatments; control, 1, 2, 3, 6 and 10% v/v effluent. The initial mean weights of all fish stocked in each of the exposure groups were similar ($p < 0.05$).

Before the effluent was introduced into the dilution system, the fish were allowed 24 h to recover from the stress of handling. Introduction of the effluent into the exposure tanks marked day one of the exposure period.

The fish were fed "Ewos" trout starter pellets four times daily at a ration level of about 8% wet weight per day except for a 24-h period before they were weighed and measured. This ration level was maintained throughout the exposure. Accumulated feces and excess food were removed from the tanks each day prior to the initial feeding.

The aquaria were artificially illuminated with a combination of fluorescent and incandescent lights for a 16 h photoperiod. A light controller

(Drummond and Dawson 1970) maintained a dawn and dusk simulation of light intensity. Growth aquaria were randomly placed in the testing area to negate any effect of position within the area. A black polyethylene sheet surrounded the growth aquaria to provide a shield against mechanical disturbances and extraneous sources of light.

For this portion of the study the proportional diluter flow rate was increased to 430 ± 20 ml per min for each aquaria and rechecked at 2-day intervals throughout the experiment. Dissolved oxygen, pH, temperature and conductivity were measured daily in each of the growth aquaria. Samples of the effluent dilutions were collected at 5-day intervals for routine chemical analysis (Appendix 3).

At the end of an 18-day exposure period the experiment was terminated due to a complete shut-down of all the mill production areas. The fish were starved 24 h prior to the termination, sacrificed with a lethal concentration of MS-222, and weighed to the nearest 0.01 g. Fork length was measured to the nearest 0.1 cm.

Initial and final mean weights for each of the duplicate populations were compared by analysis of independent t-distribution. Further statistical analysis attempted to correlate the degree of growth impairment with an increase in effluent concentration.

Sublethal Effects - Flavor Impairment

Yearling rainbow trout (≃ 200 g), purchased from the Otterville hatchery, were utilized for the study on flavor impairment. The fish were acclimated for 2 weeks at 15 ± 1°C to water pumped from Nipigon Bay. All systems for effluent collection, cooling and dilution were the same as those for all other continuous-flow bioassays. The steam stripper system was

operational throughout the exposure period. Duplicate populations of 10 fish each were exposed to final effluent concentrations of 2, 3, 6, 10 and 18% v/v plus additional control solutions. The exposure period for fish held in 3, 6, 10 and 18% effluent concentrations was 48 h while additional fish were held in 2 and 3% test solutions for 144 h. Due to the high fish loading rate in this portion of the study all bioassay containers were aerated to maintain dissolved oxygen concentrations above 7 mg/l. Constant replacement of diluted effluent in this continuous-flow bioassay minimized the potential stripping of volatile components due to the continual aeration. The fish were fed "Ewos" trout food pellets throughout the acclimation and exposure periods. At the termination of each portion of the study the fish were sacrificed by a blow to the head, sealed in polyethylene bags and quickly frozen.

The frozen fish were returned to the central laboratory for flavor evaluation. The flavor evaluation tests were conducted according to the taste panel method described by Wells (1972). The presence or absence of foreign flavor as compared with the control was recorded as follows: 0 - absent, + - barely perceptible; ++ - definite; or +++ - strong. All responses in the definite or strong categories were designated as positive responses. One unknown control was always included and several fish were duplicated during each tasting session.

The Mann-Whitney non parametric ranking test was used to compare taste panel responses to samples from each member of a pair of duplicate effluent concentrations. Chi-square analyses evaluated the incidence of foreign flavor of fish exposed to the effluent compared to the flavor of control fish.

RESULTS

Acute Bioassays - Static

Three on-site semi-static bioassays conducted prior to the installation of the steam stripper produced a mean 96-h LC50 value of 16.4 ± 2.4% v/v (Table 1). These bioassays were not aerated but there was a periodic addition of small volumes of pure oxygen. Two bioassays conducted at the central laboratory with continual aeration and no solution replacement had an increased mean LC50 figure of 36.5 ± 0.5% v/v. Aerated effluent dilutions produced a noticeable sulphurous odor for a period of 24 to 36 h, suggesting that volatile sulphur compounds were being stripped off.

Two additional semi-static bioassays were conducted on-site. The sample for the first bioassay was collected while the stripper facility was inoperative and resulted in an LC50 value of 28.0% v/v. The second bioassay conducted with the steam stripper on-line produced an LC50 of 49.0% v/v. As all other production variables of the mill were similar when the effluent samples were collected for these two bioassays, there is an indication that the steam stripper may be effective in reducing the acute toxicity of the mill effluent.

Bioassay containers aerated continuously at the central laboratory were held at >90% oxygen saturation throughout the exposure period. Dissolved oxygen levels in the on-site bioassay containers declined somewhat in the highest effluent concentrations (> 18% v/v), but as a result of a 48-h solution replacement and periodic oxygenation, the dissolved oxygen concentration was not less than 4.0 mg/l for longer than 6 h.

During each of the static and semi-static bioassays, fish in the higher (> 18% v/v) effluent concentrations displayed an initial increased respiratory rate, followed by a loss of equilibrium within the first

Table 1: Toxicity of B K M E to juvenile rainbow trout (Salmo gairdneri)

Date Tested Day/Month/Year	Treatment	Loading Rate g/l	96-h LC50 (% v/v)
27/02/74	Static, aerated[a]	0.869	37.0
09/04/74	Static, aerated[a]	0.499	36.0
27/09/73	Static, non-aerated[b]	0.825	16.5
03/07/74	Static, non-aerated[b]	0.960	14.0
09/09/74	Static, non-aerated[b]	0.750	18.7
16/06/75	Static, non-aerated[b]	0.975	28.0
24/06/75	Static, non-aerated steam stripped[b]	1.09	49.0
07/07/75	Continuous flow non-aerated steam stripped[b]	.337	24.8
14/07/75	Continuous flow non-aerated steam stripped[b]	.427	21.8

[a]tested at central laboratory

[b]tested on site

48 to 72 h.

Mean measured pH values for the highest effluent concentrations tended to be alkaline (pH 8.0) but were satisfactory for fish survival.

Acute Bioassays - Continuous-Flow

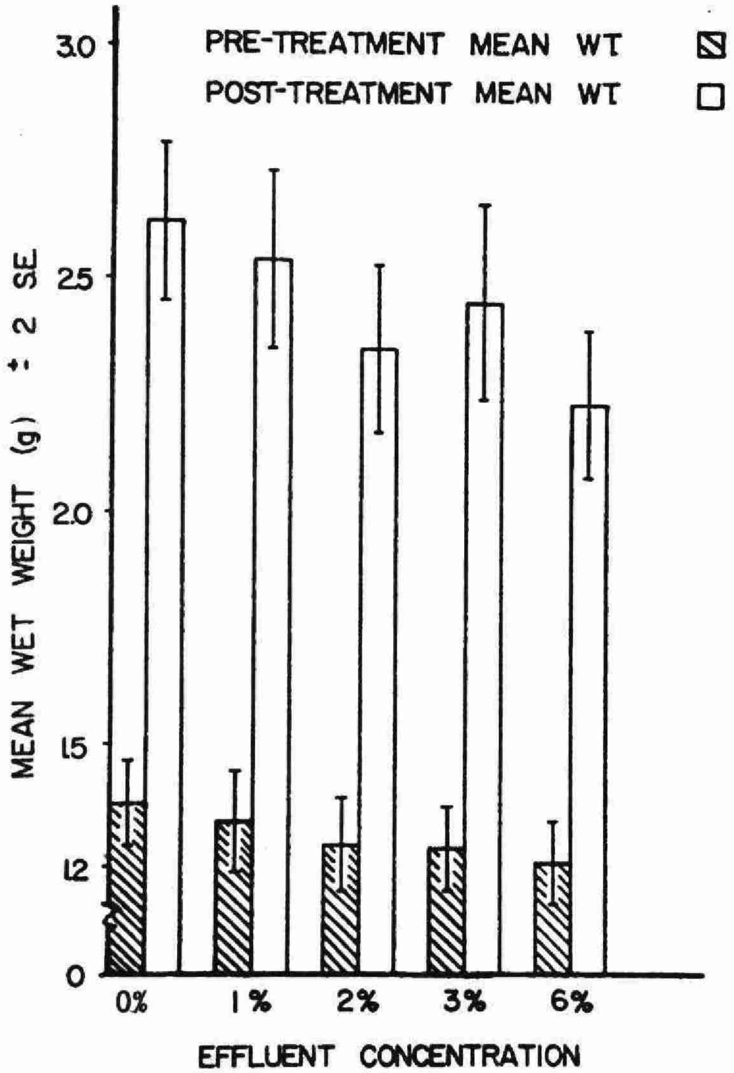
A mean 96-h LC50 of 23.3% v/v was derived from the results of two continuous-flow bioassays conducted with the steam stripper treatment system in operation. This represents an increase of >100% in the 96-h LC50 in comparison to those values derived from static bioassays conducted on similarly steam stripped effluent. Resin acid analysis of total mill effluent collected during these bioassays resulted in a mean concentration of 3.38 ± 1.03 mg/l. Isopimaric acid accounted for 34.8% of the total resin acids detected, while dehydroabietic and abietic acid constituted 11.6 and 26.6% respectively, of the resin acid content of the effluent.

No major spills or process malfunctions were reported or evident from the pH and conductivity monitoring system during the test period. The mixing action of the diluter maintained the dissolved oxygen above 80% saturation in all concentrations. No noticeable sulphurous odors were emitted from the effluent concentrations tested, contrary to observations for the aerated static bioassays. Lethality did not usually occur until after 72 h of exposure, and prior to death, fish behaved similarly to those observed in the higher static effluent concentrations.

Sublethal Effects - Growth Impairment

Mean wet weights increased in all effluent exposed groups but the changes were less than those for the control populations (Fig. 2). Growth rates of fish exposed to a 6% v/v effluent concentration were reduced

Figure 2: Mean wet weight changes of juvenile rainbow trout (Salmo gairdneri) exposed to sublethal concentrations of B K M E.



($p < 0.05$) while growth rates of fish held in 1%, 2% and 3% effluent concentrations were not significantly different from controls ($p > 0.05$). Reduced weight gain and effluent concentrations were significantly correlated ($r = -0.86$, $p < 0.05$).

All fish exhibited a rapid and apparent complete recovery from the anesthetic administered during the measurement procedure. Fish exposed to the 6% and 10% effluent concentrations showed signs of reduced feeding activity and erratic opercular ventilation after 7 days. Additional exposure to 10% effluent produced loss of equilibrium and surface breathing in fish after 10 days and there was 65% mortality after 16 days.

Three major spills were detected during the 18 day growth exposure. When a spill was reported, the waste delivery system was shut down to maintain pre-spill concentrations in the exposure tanks, and not reactivated until normal production operations had resumed.

Sublethal Effects - Flavor Impairment

Results of the taste panel responses indicated that a definite or strong taint was observed in 50% or more of the fish exposed to effluent concentrations greater than 2% v/v (Table 2). Chi-square analysis of the data indicated that after 48 h of exposure, fish in 3, 6, 10 and 18% v/v effluent concentrations showed a significant ($p < 0.05$) impairment in flavor relative to control fish (Table 3). No increase in tainting was apparent in fish exposed to 3% effluent for 144 h compared to the 48 h exposure. Fish held in 3% effluent for 48 and 144 h showed a significant ($p < 0.05$) impairment in flavor relative to fish exposed to 2% effluent for 144 h. The 3% effluent concentration represents approximately 0.12 of the 96-h continuous-flow LC50 value for the steam stripped effluent. Relative to control

Table 2: Incidence of foreign flavour in B K M E exposed rainbow trout, compared to identified control samples.

No. of Samples	Concentration	Exposure Period	Percent Positive[a]
36	Control (unidentified)	144 h	19.4
17	18%	48 h	94.1
18	10%	48 h	77.8
18	6%	48 h	50.0
24	3%	48 h	54.2
11	3%	144 h	63.6
12	2%	144 h	16.7

[a] Definite or Strong Response

Table 3: A comparison of taste panel responses to flesh samples of rainbow trout exposed to B K M E for two time periods.

Concentration (% v/v)	Exposure Period	vs	Concentration (% v/v)	Exposure Period	Level of Significance[a]
18	48 h		control	144 h	S.D.
10	48 h		control	144 h	S.D.
6	48 h		control	144 h	S.D.
3	48 h		control	144 h	S.D.
3	144 h		control	144 h	S.D.
2	144 h		control	144 h	N.S.D.
18	48 h		10	48 h	N.S.D.
10	48 h		6	48 h	S.D.
6	48 h		3	48 h	N.S.D.
6	48 h		3	144 h	N.S.D.
3	48 h		3	144 h	N.S.D.
3	48 h		2	144 h	S.D.
3	144 h		2	144 h	S.D.

[a]S.D. - significantly different ($p < 0.05$)

N.S.D. - not significantly different ($p > 0.05$)

samples no significant ($p > 0.05$) tainting was identified in fish exposed to 2% effluent for 144 h .

Although the panelists were not evaluating samples from a single fish taken from each one of the exposure concentrations, statistical analysis showed that this factor did not interfere with the interpretation of the data. Further analysis indicated that panelists could not distinguish a significant difference in flavor between fish taken from either member of a pair of duplicate effluent concentrations.

DISCUSSION

Aeration, a recognized method of BKME treatment, oxidizes sulphur compounds and this reduces the toxicity of the effluent (Werner 1963). Seppovaara and Hynninen (1970) demonstrated that volatile sulphur compounds could be readily air-stripped from the effluent with a resultant decrease in toxicity. Oxidation by air or steam stripping of BKME containing high concentrations of sulphur compounds significantly reduces the effluent toxicity, but if small quantities of volatile toxicants are present only a minimal reduction in toxicity will result.

When determining the effect of a kraft mill discharge on a local fisheries population, the receiving water conditions must be considered. If a kraft mill effluent is discharged to a well mixed receiving water, volatile toxicants will be rapidly dissipated. If the receiving water is of limited capacity with a low turnover rate, the discharge of volatile toxicants will be relatively significant. The kraft mill on Nipigon Bay discharges an effluent with a mean BOD of 16.6 tons per day. The high oxygen demand of this effluent may deplete the dissolved oxygen level of the receiving water in an area immediately adjacent to the outfall. Thus during periods

of low flow and lowered oxygen levels non-aerated bioassays may be more relevant in assessing the toxicity of the effluent at that time.

Prior to the installation of the steam stripper, mean measured hydrogen sulfide levels for total mill effluent samples were 6.8 mg/l, an order of magnitude greater than the 120-h LC50 of 0.7 mg/l H_2S for cutthroat trout (Salmo clarki) as reported by Haydu et al (1952). After the stripper was installed, hydrogen sulfide levels were nondetectable in the total mill effluent while sulfate concentrations were reduced by 50% to 100 mg/l, the minimum lethal concentration reported by Haydu et al (1952) for a species of minnow.

The toxic action of sulfur compounds may involve respiratory failure and suffocation. Hydrogen sulfide binds to the metal constituents of enzymes and hence inhibits the iron containing cytochrome C-enzyme in the respiratory chain (Werner 1963). Seppovaara and Hynninen (1970) stated that toxic effects of sulfides and mercaptans result in the reduced ability of red blood cells to transport oxygen. In all bioassays conducted with and without continued aeration, dissolved oxygen content of the effluent dilutions did not fall below 4.0 mg/litre. Visual observations of fish in lethal concentrations indicated severe respiratory stress prior to death. Therefore, a portion of the toxicity of the effluent may be attributed to the inability of the fish to utilize available oxygen.

Although no deaths in the higher effluent concentrations could be attributed directly to anoxia, the stress placed upon the fish due to diminished oxygen levels for portions of the test period would decrease their tolerance to other toxicants present in the waste.

Leach and Thakore (1973) identified some of the non volatile

constituents of a kraft pulping effluent that were acutely toxic to juvenile coho salmon (Oncorhynchus kisutch). They determined that 80% of the effluent's toxicity was attributable to resin acid soaps. Mean resin acid levels measured in lethal effluent concentrations of continuous flow bioassays were within the lethal threshold range of 1 to 3 mg/litre (Leach and Thakore 1973).

A comparison of the results derived from continuous-flow and on-site static bioassays with 48-h solution replacement indicates that the continuous-flow method consistently yielded higher toxicity values. Loch and MacLeod (1974), investigating the acute lethality of several kraft mill effluents, indicated that flow-through bioassays appeared to be at least fourfold more sensitive than static bioassays. Results from the Nipigon Bay study suggest that continuous-flow bioassays are more than twice as sensitive as "semi-static" (48-hour solution replacement) bioassays even when the steam stripper facility is operating. Continuous-flow bioassays are most costly to set up, require considerably more effluent than static bioassays, and necessitate on-site testing, but they provide a more representative profile of kraft mill effluent toxicity. The continual solution replacement of flow-through bioassay reduces the extent to which the toxicants may be depleted by volatilization and adsorption.

Responses of fish to sublethal levels of contaminants, such as growth impairment or the production of an unnatural flavor in fish flesh, are critical to the existence of a fishery or the marketability of a commercial fishery.

The mean weight increase of fish in exposure tanks receiving 3% v/v BKME was less than but not significantly different from the weight increase observed for the control groups but greater than that of fish held in 2%

and 6% effluent concentrations. This result suggests that a slight growth stimulation may have occurred in the 3% effluent concentration. Other authors have observed growth reduction and stimulation in salmonids after exposure to low concentrations of BKME (Table 4). Warren and Davis (1967) suggested that a reduction in growth rate on exposure to sublethal concentrations of BKME may be a result of a decrease in either food intake, assimilation efficiency, metabolic processing or an increase in maintenance requirements. A preliminary study by Webb and Brett (1972) demonstrated that oxygen consumption was elevated by a factor of about two in effluent concentrations as low as 1% of the 24-h LC50. Thus, it has been postulated that exposure to sublethal concentrations of BKME may result in elevation of maintenance energy requirements and thus reduce the proportion of energy available for growth.

Tokar and Owens (1968) and McLeay and Brown (1974) have reported growth stimulation in salmonids on exposure to low effluent concentrations of from 0.07 to 0.25 of the 96-h LC50 value. They have suggested that the dark colour of the effluent may reduce interaction between fish, thus decreasing aggression and reducing the maintenance requirements of the fish. This would increase the energy available for growth. McLeay and Brown (1974) stated that kraft mill effluents may contain minimal amounts of growth increasing factors (hormone analogs) derived from the wood extractives. These authors also discussed the possibility that the salinity of certain effluent concentrations provided optimum osmotic conditions for maximum growth.

The extent of a fisheries' tainting problem in an area is difficult to quickly assess since the tainted fish appear normal on gross inspection.

Table 4: Effect of kraft mill effluents on growth rate of salmonids.

Author	Species	Effluent Type	Concentration v/v	% 96-h LC50	Exposure Period (days)	Growth Rate Effect
Webb & Brett	Sockeye salmon	B K M E [a]	25%	(-)	57	Reduced
Webb & Brett (1972)	Sockeye salmon	B K M E	1.0 & 2.5%	(-)	57	No effect
Tokar & Owens (1968)	Chinook salmon	U K M E [b]	-	(0.09 - 0.07)	14-18	Increased
Davis & Mason (1973)	Coho salmon	B K M E	-	(0.2 - 0.05)	50	Increased
McLeay & Brown (1974)	Coho salmon	B K M E	-	(0.25 - 0.1)	50	No effect
McLeay & Brown (1974)	Coho salmon	B K M E	-	(0.25)	200	Increased

[a] B K M E - Bleached kraft mill effluent

[b] U K M E - Unbleached kraft mill effluent

Organoleptic testing is the only proven means of accurately defining the degree of flavor impairment. Whole fish may be used for flavor evaluation (Shumway 1968) or samples may be taken from specific areas of the fish (Wells 1970 and Swabey 1964). Baldwin et al. (1969) could not detect any flavor difference in samples taken from various areas of the body.

Tainting compounds may originate from either natural sources or industrial discharges. Thaysen and Pentelow (1936) reported that a type of Actinomyces could produce an "earthy" pungent odor in water and result in the tainting of fish. The results of the threshold odor determinations conducted on the Nipigon Bay dilution water (Appendix 1) suggests that the tainting observed was not a result of natural sources.

Results of the Nipigon Bay study indicate that once a fish has become tainted, an increase in the length of the exposure period at that concentration does not seem to intensify the taint. Also, if an effluent concentration does not taint the fish in a short time period, increasing the exposure period threefold will not impair flavor.

The fish flavor impairment evaluation was found to be a more practical sublethal test than that of the fish growth evaluation due to its shorter test duration. Pulp and paper mills are prone to intermittent process upsets which result in spills potentially high in toxic compounds, plus frequent plant shutdowns which interrupt long term biological monitoring programs. The growth evaluation suffered to a certain degree on both of these counts and had to be terminated after 18 days, whereas the flavor impairment evaluation exhibited a more sensitive response within 48 hours of its initiation.

It must be kept in mind that the taste test and growth study were not run concurrently, nevertheless a comparison of their respective sensitivities is not out of line when the acute toxicity of both test effluents is taken into account. Three percent v/v effluent, the lowest concentration

impairing flavor of yearling rainbow trout after a 48 h exposure, represented less than .17 of the 48 h LC_{50} for that test; while growth impairment occurred at greater than .60 of the 18 day LC_{50} for that test.

Cook et al. (1971) demonstrated the tainting threshold of a kraft mill employing a steam stripper varied between 0.5 and 0.3 of the incipient LC_{50} which is in the same order of magnitude indicated in this study (0.14 of 96-hr. LC_{50}). The most sensitive sublethal response measured in this study, flavor impairment at a 3% v/v effluent concentration, may be the most critical response when assessing the economic impact of a kraft mill discharge to a freshwater ecosystem.

When the results of this study are applied to plume chemistry and fish distribution findings, an assessment can be made regarding the effect of the plume on the fisheries of Nipigon Bay.

The initial dilution of the mill effluent is rapid usually giving relative concentrations less than 25% v/v at the end of the discharge channel (Minns, pers. comm.). The effluent plume remained on the surface (Polak, 1974), 1-2 metres thick and seldom traversed depths greater than 10 metres (Minns, 1975). Therefore, under normal mill operating conditions it is unlikely that effluent concentrations approach lethal levels in Nipigon Bay beyond the immediate area of the outfall.

An area of 1.5 km^2 of Nipigon Bay is exposed to concentrations of pulp and paper mill effluent greater than 1% based on dispersal of the conservative sodium ion (Minns, 1975). Therefore, the zone of tainting which would occur in Nipigon Bay due to the bioconcentration of flavor impairing compounds by fish encompasses an area of less than 1.5 sq. km.

However, due to the fact that there is an intense aggregation of fish within 1 km of the discharge (Kelso, 1975) and benthic fauna do exist in much of the area influenced by the mill discharge (Vander Wal, 1975), the most probable mechanism for flavor impairment of fish in Nipigon Bay is bioaccumulation of flesh impairing contaminants through the food chain.

CONCLUSIONS

Under normal mill operating conditions it is unlikely that effluent concentrations would approach lethal levels beyond the immediate area of the outfall to Nipigon Bay.

The mill effluent has the potential to reduce the growth rates of fish.

The zone of tainting which would occur in Nipigon Bay due to the bioconcentration of flavor impairing compounds by fish encompasses an area of less than 1.5 sq. km. However, other transport mechanisms, such as the bioaccumulation of contaminants from effluent-exposed organisms, may extend the mill's zone of influence.

Although continuous-flow bioassays are more costly, require more effluent than static bioassays and necessitate on-site testing, they do provide a more representative profile of effluent toxicity.

The fish flavor impairment evaluation was found to be a more sensitive and practical on-site sub-lethal test than that of a fish growth evaluation. In fact, a flavor evaluation may also provide the most critical response when assessing the economic impact of an industrial discharge to a freshwater system.

RECOMMENDATIONS

Domtar Packaging Limited should remove or reduce all substances in their final effluent which produce or combine to produce a significant effect on fish lethality, growth or the tainting of fish flesh.

Emphasis of future research on pulp and paper mill effluents should be directed toward the bioaccumulation and persistence of trace organics in invertebrates and indigenous fish species, as well as rainbow trout. Some of these compounds are highly toxic and have the potential to taint fish available for both commercial and recreational fishing.

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APPENDICES

Appendix 1: Chemical and physical characteristics of central laboratory and Nipigon Bay dilution water.

Appendix 2: Total mill effluent characteristics for acute static and continuous-flow bioassays.

Appendix 3: Chemical and physical characteristics of effluent dilutions measured during the growth study.

Appendix 1. Mean values (±2 SD) of the chemical and physical characteristics for the central laboratory and Nipigon Bay dilution water.

Parameter	Central Laboratory Dechlorinated Tap Water	Nipigon Bay
Alkalinity as mg.$CaCO_3$/l	89 ± 3.0	68.2 ± 10
EDTA Hardness as mg$CaCO_3$/l	139 ± 5.0	69 ± 5.0
Chloride (mg/l)	33 ± 2.0	1.5 ± 0.7
Sulphate (mg/l)	33 ± 3.0	2.7 ± 1.0
Sodium (mg/l)	13 ± 1.0	1.7 ± 05
Potassium (mg/l)	1.7 ± 0.6	-
Conductivity (μmhos/cm)	332 ± 15	100.3 ± 6
pH	7.4 ± 0.2	7.8 ± 0.4
Threshold Odor	-	12.3 ± 1.3
Residual Chlorine (μg/l)	30.1 ± 3.0	-

Appendix 2. Mean values (±2 SD) of the total mill effluent characteristics for acute static and continuous-flow bioassays.

Parameter[a]	Static	Continuous Flow
Na+	152.9 ± 62.4	115.0 ± 8.6
Cl^-	88.3 ± 69.0	58.3 ± 24.9
$SO_4^=$	151.7 ± 41.3	120.0 ± 26.5
Phenol (μg/l)	807.0 ± 440.0	877.0 ± 109.0
Dissolved Solids	720.1 ± 261.7	606.7 ± 27.5
Suspended Solids	96.3 ± 52.9	66.7 ± 24.7
Alkalinity	182.1 ± 78.3	123.7 ± 9.1
BOD_5	183.3 ± 76.7	140.0 ± 10.0
COD	542.2 ± 240.1	435.0 ± 63.8
pH	8.36 ± 1.05	8.01 ± 0.35
Conductivity (μmhos/cm @ 25°C)	711.3 ± 180.9	700.0 ± 100.0
Flow ($m^3 min^{-1} \times 10^5$)	3.2 ± 0.9	3.4 ± 0.6
Na_2SO_4 (tons/day)	39.9 ± 5.7	42.9 ± 7.5
BOD_5 (tons/day)	16.6 ± 2.7	-
Suspended Solids (tons/day)	6.8 ± 0.8	6.9 ± 1.4
Dissolved Solids (tons/day)	53.6 ± 7.7	57.2 ± 9.2

[a]All results reported as mg/l unless otherwise noted.

Appendix 3. Mean values of the chemical and physical characteristics of effluent dilutions measured during growth study

Parameter[a] / Concentration of effluent in control water (v/v)	$S^=$	BOD_5	COD	Solids Susp.	Solids Diss.	Phenols (μg/l)	Alkalinity $mgCaCO_3/l$	Hardness $mgCaCO_3/l$	Conductivity (μmhos/cm)	pH	Dissolved Oxygen	Temperature (°C)
0%	0.02	1.3	23	80	2	2.3	66.5	67	134.4	7.54	9.62	15.48
1%	0.02	2.0	25	82	3	6.5	68	69	140.1	7.61	9.55	15.04
2%	0.035	3.0	15	90	6	10	70	70	142.7	7.64	9.43	14.85
3%	0.05	3.8	33	100	6	17	69.5	69	152.5	7.75	9.55	14.81
6%	0.06	5.3	25	105	8	27	72	71	158.0	7.84	9.44	15.21
10%	0.17	8.0	43	126	12	37.5	74	77	186.8	7.95	9.32	15.41

[a]All results expressed as mg/l unless otherwise noted.